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ARTICLE

Pharmacokinetic-pharmacodynamic model of urinary δ-aminolevulinic acid reduction after givosiran treatment in patients with acute hepatic porphyria

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Abstract

Givosiran, an RNA interference-based therapeutic, is a recent addition to the limited treatment armamentarium for acute hepatic porphyria (AHP). As a small interfering RNA that is selectively taken up in the liver, both the mechanism and targeted delivery create a complex relationship between givosiran pharmacokinetics (PK) and the pharmacodynamic (PD) response. Using pooled data from phase I–III clinical trials of givosiran, we developed a semimechanistic PK/PD model to describe the relationship between predicted liver and RNA-induced silencing complex concentrations of givosiran and the associated reduction in synthesis of δ-aminolevulinic acid (ALA), a toxic heme intermediate that accumulates in patients with AHP, contributing to disease pathogenesis. Model development included quantification of variability and evaluation of covariate effects. The final model was used to assess the adequacy of the recommended givosiran dosing regimen across demographic and clinical subgroups. The population PK/PD model adequately described the time course of urinary ALA reduction with various dosing regimens of givosiran, the interindividual variability across a wide range of givosiran doses (0.035–5 mg/kg), and the influence of patient characteristics. None of the covariates tested had a clinically relevant effect on PD response that would necessitate dose adjustment. For patients with AHP, including adults, adolescents, and patients with mild to moderate renal impairment or mild hepatic impairment, the 2.5-mg/kg once monthly dosing regimen of givosiran results in clinically meaningful ALA lowering, reducing the risk for AHP attacks.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Givosiran is a synthetic, chemically modified, double-stranded, small interfering RNA (siRNA) approved for the treatment of acute hepatic porphyria (AHP). Due to the unique mechanistic features of siRNA-based therapeutics and specific targeting of delivery to the liver, the relationship between the pharmacokinetics (PK) and pharmacodynamics (PD) of givosiran is complex.

WHAT QUESTION DID THIS STUDY ADDRESS?

This work sought to develop a PK/PD model that describes the exposure-response relationship for givosiran.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Development of the semimechanistic PK/PD model provides insights that are valuable in understanding the PK/PD relationship of givosiran and, more broadly, other N-acetylgalactosamine conjugated siRNA-based therapeutics. Simulations derived from the model demonstrated that the approved givosiran dose, 2.5 mg/kg once monthly, is predicted to provide meaningful PD effects across a broad range of patients with AHP.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

This analysis provides a framework that can be applied not only to understanding the givosiran exposure-response relationship, but also to the future development of PK/PD models for siRNA-based therapeutics.

INTRODUCTION

Givosiran, a synthetic, chemically modified, double-stranded, small interfering RNA (siRNA), is approved for the treatment of acute hepatic porphyria (AHP). RNA interference (RNAi) therapeutics utilize an endogenous mechanism for regulating gene expression, wherein siRNA binds to the RNA-induced silencing complex (RISC), which directs cleavage of target messenger RNA (mRNA), resulting in gene-specific silencing.[1–3] Givosiran targets hepatic *aminolevulinic acid synthase 1 (ALAS1)* mRNA, thereby reducing synthesis of ALAS1 protein.[4,5] In patients with AHP, expression of ALAS1—the enzyme involved in the initial and rate-limiting step for heme biosynthesis—is upregulated in response to insufficient hepatic heme production caused by inherited deficiency of enzymes within the heme biosynthesis pathway.[6–8] Increased ALAS1 activity leads to accumulation of toxic heme intermediates, δ-aminolevulinic acid (ALA) and porphobilinogen (PBG), which are implicated in the pathogenesis of AHP-related acute neurovisceral attacks as well as other chronic disease manifestations.[8–12]

As the metabolic dysfunction that underpins AHP occurs in the liver, givosiran is designed for preferential hepatic uptake after subcutaneous (s.c.) administration.[4,13] The siRNA is conjugated to a trivalent N-acetylgalactosamine (GalNAc) moiety that specifically binds to asialoglycoprotein receptors (ASGPRs), which are primarily and abundantly expressed by hepatocytes.[13,14] As a result, plasma exposures for givosiran and its equipotent active metabolite, AS(N-1)3′ givosiran, are short lived, with plasma concentrations declining to below the lower limit of quantification (LLOQ) within 24 h after s.c. administration.[15] In contrast, the pharmacodynamic (PD) effects of givosiran (e.g., reduction of urinary ALA and PBG concentrations) last for days after a single s.c. dose.[5,15–17] In the pivotal, phase III clinical trial, s.c. givosiran 2.5 mg/kg administered once monthly (q.m.) produced rapid and sustained urinary ALA and PBG lowering and significantly reduced the rate of porphyria attacks versus placebo, the primary end point of the study.[16]

The duration of ALA and PBG lowering after givosiran administration does not align with the observed transient plasma exposure, indicating that the PD effects of givosiran are dependent on exposure in the liver rather than circulating drug concentrations. To better understand how givosiran pharmacokinetics (PK) relate to its PD effects, we have developed a model that describes the relationship between predicted liver and RISC concentrations of givosiran in humans using allometrically scaled nonclinical data and the associated reduction in synthesis of ALA. This semimechanistic PK/PD model linking the predicted concentrations of givosiran in human liver and RISC to the PD effect is the first of its kind and is different from previously published PK/PD models of other siRNAs that link transient plasma siRNA concentrations to PD effect.[18,19] The goals of this analysis were to characterize the exposure-response relationship in patients with AHP, quantify variability, and evaluate relevant covariate effects on the PK/PD relationship. The final model was then used to assess the adequacy of the recommended givosiran dosing regimen across demographic and clinical subgroups.

METHODS

Data sources

This analysis included pooled data from all study participants in three clinical trials (ALN-AS1-001, ALN-AS1-002,

and ALN-AS1-003) who received at least one dose of givosiran or placebo (Table S1). ALN-AS1-001 (NCT02452372) was a phase I, randomized, placebo-controlled study conducted in three parts. Parts A and B enrolled chronic high excreters (CHEs)—individuals who carry genetic mutations associated with AHP and who have elevated concentrations of ALA and/or PBG but who do not demonstrate disease manifestations associated with AHP.[7] Participants in ALN-AS1-001 Part A received a single s.c. dose of givosiran (0.035, 0.1, 0.35, 1, or 2.5 mg/kg) or placebo and participants in Part B received two doses of givosiran (0.35 or 1.0 mg/kg) or placebo q.m. In ALN-AS1-001 Part C, patients with symptomatic acute intermittent porphyria (AIP) were randomized 3:1 to receive givosiran (2.5 or 5.0 mg/kg) or placebo q.m. or every 3 months (q3m). Patients who completed ALN-AS1-001 Part C were eligible for the phase I/II, open-label extension study, ALN-AS1-002 (NCT02949830). All patients in ALN-AS1-002 were initially assigned to receive givosiran 2.5 or 5.0 mg/kg q.m. or 5 mg/kg q3m; subsequently, the protocol was amended to make dosing uniform for all patients (2.5 mg/kg q.m.; the dose of givosiran used in the phase III clinical trial). The phase III, double-blind, placebo-controlled study, ALN-AS1-003 (ENVISION; NCT03338816), randomized patients with AHP 1:1 to treatment with givosiran 2.5 mg/kg or placebo q.m. for a period of 6 months. Patients were then eligible for an open-label extension during which they received givosiran 2.5 or 1.25 mg/kg q.m. All patients transitioned to givosiran 2.5 mg/kg after the month 13 visit. The open-label extension study was ongoing at the time of this analysis and is now complete[20]; the majority of data included in this PK/PD analysis was from the 6-month, double-blind treatment period of the phase III study.

For all studies, protocols were approved by ethics committees or institutional review boards at respective participating centers and written informed consent was obtained from all participants. Details of the study inclusion/exclusion criteria and study design for the phase I and III studies have been previously published.[5,15,16]

Pharmacodynamic sampling

Urinary concentrations of both ALA and PBG were measured in givosiran clinical trials. Previous data have shown strong correlations between plasma concentrations of ALA and PBG and their urinary counterparts in subjects with CHE or AIP.[15] As demonstrated using data from ALN-AS1-001, urinary concentrations of ALA and PBG are also highly correlated (Pearson correlation coefficient = 0.7771; Figure S1). As such, incorporation of both biomarkers in the model was not expected to provide any additional insights into the givosiran PK/PD relationship. Therefore, ALA, which scientific and clinical evidence suggests is the main contributor to disease manifestations in AHP,[9,11,21] was used as the sole PD effect measure in PK/PD model development. Urinary concentrations of ALA were determined using a sensitive and validated liquid-chromatography tandem mass spectrometry assay with an LLOQ of 10 ng/mL. Creatinine (Cr) concentrations were determined using standard laboratory assays and were used to normalize ALA levels in urine. The timing of urinary ALA sampling by study is listed in Table S1.

Prediction of human liver PK from nonclinical data

As it is not practical to measure liver PK in humans, givosiran PK in human liver and RISC were predicted from nonclinical studies[13,22] using allometric scaling. For the purposes of PK modeling, liver concentrations of active siRNA (givosiran and equipotent metabolite, AS(N-1)3′ givosiran, combined) in rats were parameterized to a two-compartment model. RISC concentrations of active siRNA in rats were parameterized to a one-compartment model with first-order absorption from the peripheral liver compartment. Liver PK for a typical 70-kg subject was predicted based on allometric scaling of parameters from the liver PK model in rats with exponents of one for volume of distribution (V_d) and 0.75 for clearance (CL). The uptake rate constant of active siRNA into human liver was assumed to be similar to rats, with an absorption half-life of ~0.24 h. The predicted apparent V_d values for active siRNA in human liver are 6.36 kg (V_1) and 5.21 kg (V_2), with predicted liver CL of 13.3 g/h, an alpha elimination half-life of 12.2 days, and a beta terminal elimination half-life of 122 days.

Development of the population PK/PD model

The PK/PD model used a semimechanistic model linking the predicted concentrations of active siRNA in human liver to the PD effect, urinary ALA reduction, through an intermediate compartment that represented concentrations of active siRNA in RISC. Model building included development of the structural model and development of the random-effects model, including interindividual variability (IIV) and residual variability. A first-order conditional estimation method with interaction was used throughout the model development process. The structural PK/PD model was selected based on statistical criteria (e.g., minimum objective function

value [OFV], condition number) as well as pertinent graphical representations of goodness-of-fit (e.g., predicted and observed ALA vs. time, conditional weighted residuals vs. time). IIV was modeled using exponential random effects models and proportional and additive residual variabilities were also assessed.

Intravenous hemin for treatment of acute attacks was permitted in givosiran clinical trials. Hemin treatment leads to transient lowering of elevated urinary ALA levels and alleviation of attack symptoms, an effect that lasts for 2–5 days.[23,24] Due to the potential confounding influence of hemin administration on ALA lowering with givosiran, the effect of hemin was incorporated into the PK/PD model.

Covariate model development

The relationships between potential covariates and PD parameters were explored graphically. Scatter plots for continuous variables and box plots for categorical variables were used to describe the relationship between potential covariates and interindividual random effects of parameters derived from the structural model. The effects of predefined covariates (Table S2) on PD parameters were evaluated using a full model approach, wherein all covariates were included in the model simultaneously, with care to avoid simultaneous inclusion of correlated covariates. The full covariate model was simplified to the reduced model by retaining clinically significant predefined covariates and eliminating covariate effects in which the 95% confidence interval included one. Exploratory covariates were retained in the final model if the goodness-of-fit was meaningfully improved (change in OFV of ≥10.8 units for 1 degree of freedom, $\alpha = 0.001$).

Model selection and evaluation

The final population PK/PD model was evaluated using graphical representations of goodness-of-fit and visual predictive checks. Based on the estimates of the final model, urinary ALA level-time profiles were simulated using 500 replicates. Observed and simulated data were separated into bins (e.g., nominal time). Within each bin, nonparametric 95% prediction intervals for the 10th, 50th, and 90th percentiles of predicted concentrations were computed and compared with the 10th, 50th, and 90th percentiles of observed data.

Model application

Based on the estimates of the final population PK/PD model, urinary ALA levels were simulated (500 replicates to include IIV) at steady-state based on a givosiran dose of 2.5 mg/kg q.m. in a simulation population with significant covariates. The relationship between givosiran dose and urinary ALA for q.m. dosing at steady-state in patients with AHP was evaluated. The model was also used to predict ALA level-time profiles for q.m. versus q3m dosing and for various patient body weights. Comparison of urinary ALA reduction in adolescents (age ≥12 to <18 years) and adults (age ≥18 years) was modeled by predicting ALA reduction in a 66.2-kg (adult) versus a 40-kg (adolescent) individual following s.c. givosiran 2.5 mg/kg q.m.

The population PK/PD analysis was performed using nonlinear mixed effects modeling software, NONMEM version 7.4.1 (ICON plc, Gaithersburg, MD). Processing and graphical analysis of data at each modeling step was conducted using R (version 3.5.3).

RESULTS

Analysis dataset

The pooled analysis population included 23 (17.2%) participants with CHE and 111 (82.8%) patients with AHP. A total of 2600 measurable urinary ALA samples from 134 subjects in the givosiran and placebo treatment groups were available for PK/PD modeling. The phase III study represented 47.4% of the overall urinary ALA samples included in the analysis.

Study subjects were predominantly women (87.3%) and White (81.3%). The median (range) age of the study population was 38.0 (19.0, 65.0) years and the median body weight (range) was 66.2 (39.5, 131.3) kg (Table 1). Median baseline urinary ALA level was approximately two-fold higher in patients with AHP (15.8 mmol/mol Cr) compared with participants with CHE (6.80 mmol/mol Cr). Thirteen (9.7%) subjects had hepatic impairment; 11 of the cases were mild, one was moderate, and one was severe based on National Cancer Institute Organ Dysfunction Working Group classification; however, all were grouped in the "mild" category for the purposes of analysis. Mild, moderate, or severe renal impairment was present in 49.3%, 26.9%, and 0.7% of subjects, respectively.

Structural PK/PD model

Development of the structural PK/PD model was driven by mechanistic hypotheses, statistical considerations, and heuristics guided by observed data trends. The structural PK/PD model is illustrated schematically in Figure 1.

TABLE 1 Baseline demographics and patient characteristics by study.

Baseline characteristics	ALN-AS1-001[a] A/B (*N* = 23 CHE)	ALN-AS1-002[b] (*N* = 17 AIP)	ALN-AS1-003 (*N* = 94 AHP)	Total (*N* = 134)
Female sex, *n* (%)	18 (78.3)	15 (88.2)	84 (89.4)	117 (87.3)
Age,[c] years	47 (30, 64)	39 (21, 60)	37.5 (19, 65)	38 (19, 65)
Weight,[c] kg	75 (57.3, 118)	73.1 (44.5, 118.4)	66 (39.5, 131.3)	66.2 (39.5, 131.3)
Race				
White	22 (95.7)	14 (82.4)	73 (77.7)	109 (81.3)
Asian	1 (4.3)	1 (5.9)	15 (16.0)	17 (12.7)
Other	0	2 (11.8)	6 (6.4)	8 (6.0)
Ethnicity, *n* (%)				
East Asian	0	0	12 (12.8%)	12 (9%)
Non-East Asian	23 (100%)	17 (100%)	82 (87.2%)	122 (91%)
Clinical subgroup, *n* (%)				
CHE	23 (100%)	0	0	23 (17.2%)
AHP	0	17 (100%)	94 (100%)	111 (82.8%)
Baseline ALA,[c] mmol/mol Cr	6.8 (2.5, 23.6)	15.6 (1.5, 50.5)	15.8 (0.7, 88.9)	15.3 (0.7, 88.9)
Hepatic function category, *n* (%)				
Normal	21 (91.3%)	16 (94.1%)	84 (89.4%)	121 (90.3%)
Mild hepatic impairment	2 (8.7%)	1 (5.9%)	8 (8.5%)	11 (8.2%)
Moderate hepatic impairment[d]	0	0	1 (1.1%)	1 (0.7%)
Severe hepatic impairment[d]	0	0	1 (1.1%)	1 (0.7%)
Baseline eGFR,[c] mL/min/1.73 m^2	76.2 (51.1, 141.2)	71.2 (38.5, 126.2)	67 (26, 151)	70.4 (26, 151)
Renal function category, *n* (%)				
Normal	6 (26.1%)	4 (23.5%)	21 (22.3%)	31 (23.1%)
Mild renal impairment	12 (52.2%)	8 (47.1%)	46 (48.9%)	66 (49.3%)
Moderate renal impairment	5 (21.7%)	5 (29.4%)	26 (27.7%)	36 (26.9%)
Severe renal impairment[e]	0	0	1 (1.1%)	1 (0.7%)

Abbreviations: AHP, acute hepatic porphyria; AIP, acute intermittent porphyria; ALA, aminolevulinic acid; CHE, chronic high excreter; Cr, creatinine; eGFR, estimated glomerular filtration rate.

[a]Three participants with CHE were included in both Parts A and B; two participants were included in two different treatment groups in Part A.

[b]ALN-AS1-002 patients were from ALN-AS1-001 Part C; four of 16 patients were treated with placebo in ALN-AS1-001.

[c]Data are medians (ranges).

[d]Two patients (1 with moderate and 1 with severe hepatic impairment) were pooled in the mild hepatic impairment category for the covariate analysis.

[e]The single patient with severe renal impairment was pooled in the moderate renal impairment category for covariate analysis.

The effect of allometrically scaled concentrations of active siRNA in the liver on urinary ALA levels was modeled as an inhibitory effect on the synthesis rate of ALA through an intermediary RISC effect compartment, as follows:

$$\frac{\mathrm{dALA}}{\mathrm{d}t} = k_{\mathrm{in,ALA}} \times \text{Givosiran Effect} \times \text{Hemin Effect} - k_{\mathrm{out,ALA}} \times \mathrm{ALA}$$

where $k_{\mathrm{in,ALA}}$ is the zero-order formation rate of urinary ALA and $k_{\mathrm{out,ALA}}$ is the first-order degradation rate constant of urinary ALA.

The effect of givosiran was modeled using inhibitory effect model ($I_{\max}$) on $k_{\mathrm{in,ALA}}$

$$\text{Givosiran Effect} = \left(1 - I_{\mathrm{max,givo}} \cdot \frac{C_{\mathrm{RISC}}(t)}{\mathrm{IC}_{50,\mathrm{givo}} + C_{\mathrm{RISC}}(t)}\right)$$

where $C_{\mathrm{RISC}}(t)$ is the RISC concentration of active siRNA at time "t" predicted with a PK model. $I_{\mathrm{max,givo}}$ represents maximum inhibition effect of active siRNA on $k_{\mathrm{in,ALA}}$ and $\mathrm{IC}_{50,\mathrm{givo}}$ represents the RISC concentration of active siRNA reaching 50% of maximum inhibition of givosiran.

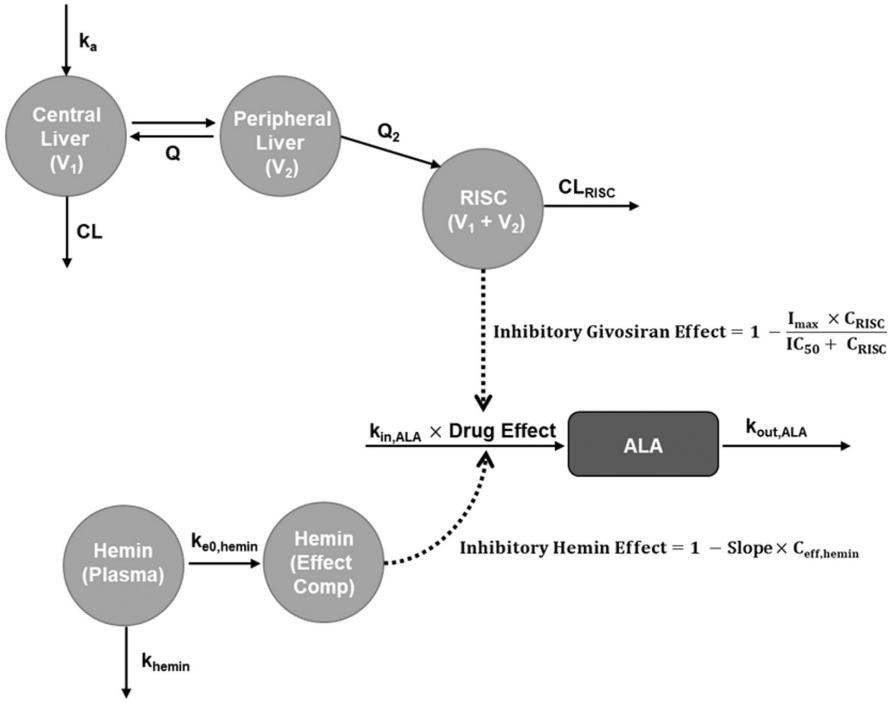

FIGURE 1 Schematic representation of the population pharmacokinetic/pharmacodynamic model for aminolevulinic acid in humans. ALA, urinary aminolevulinic acid compartment; $C_{\text{eff,hemin}}$, hemin concentration of effect compartment; C_{RISC}, RNA-induced silencing complex (RISC) concentration of active small interfering RNA; CL, clearance; CL_{RISC}, clearance from RISC compartment; IC_{50}, concentration required to produce half-maximal effect of givosiran; $I_{\max}$, maximum inhibitory effect of givosiran on $k_{\text{in,ALA}}$; k_a, uptake rate constant to liver; $k_{\text{e0,hemin}}$, effect compartment equilibrium rate constant of hemin; k_{hemin}, elimination rate constant for hemin; $k_{\text{in,ALA}}$, zero-order synthesis rate of ALA; $k_{\text{out,ALA}}$, first-order degradation rate constant for ALA; Q, intercompartmental clearance; Q_2, turnover rate of givosiran from peripheral liver compartment into RISC; V_1, volume of distribution of central liver compartment; V_2, volume of distribution of peripheral liver compartment.

An additive effect of hemin was assumed in patients with AHP who received hemin during clinical studies and was included in the model as an additional inhibitory effect on the synthesis rate of ALA.

$$\text{Hemin Effect} = \left(1 - \text{Slope} \cdot C_{\text{eff,hemin}}(t)\right)$$

where $C_{\text{eff,hemin}}(t)$ is the effect compartment concentration of hemin at time "t" predicted with a literature-reported value for the elimination rate constant "k_{hemin}" of $0.0642\,\text{h}^{-1}$ (the half-life of hemin is ~10.8 h).[25]

Clinical subgroup (CHE or AHP) was a significant covariate (OFV decrease, 36.87) on $k_{\text{in,ALA}}$ and $\text{IC}_{50,\text{givo}}$ and was included in the structural PK/PD model. Participants with CHE are predicted to have lower baseline ALA levels (~58% lower) and lower $\text{IC}_{50,\text{givo}}$ values (~70% lower) than patients with AHP based on the longer duration of greater than 50% ALA lowering observed following a single dose of givosiran 2.5 mg/kg in subjects with CHE versus AHP.

Covariate analysis

The full covariate model that incorporated predefined covariates converged successfully with reasonable parameter estimates (Table 2). In addition to covariates already included in the structural PK/PD model, baseline ALA and age were found to be significant covariates on $I_{\text{max,givo}}$ and mild hepatic impairment was a significant covariate on $k_{\text{in,ALA}}$; these covariates were retained in the model. Exploratory covariates listed in Table S2 were tested; none resulted in a statistically significant change in OFV. Four covariates were retained in the final PK/PD model: clinical subgroup (CHE vs. AHP) on $\text{IC}_{50,\text{givo}}$ and $k_{\text{in,ALA}}$, mild hepatic impairment on $k_{\text{in,ALA}}$, and baseline ALA on $I_{\text{max,givo}}$.

Final PK/PD model

Typical values of $k_{\text{in,ALA}}$ were estimated as 5.312 and 11.47 mmol/mol Cr·h for subjects with CHE and AHP, respectively (Table 2). The typical value of $k_{\text{out,ALA}}$ was fixed as $0.84\,\text{h}^{-1}$, which reflects an ALA degradation half-life of 0.825 h.[26] The ratios of $k_{\text{in,ALA}}$ to $k_{\text{out,ALA}}$ were 6.324 and 13.65 mmol/mol Cr for subjects with CHE and patients with AHP, respectively, which were consistent with the observed baseline urinary ALA levels. The hemin effect on ALA was incorporated with a linear function related to hemin concentration in the effect compartment. The first-order rate

TABLE 2 Parameter estimates for the structural, full, and final population PK/PD models.

	Structural Model		Full model		Final model	
Parameter	**Estimates**	**RSE, %**	**Estimates**	**RSE, %**	**Estimates**	**RSE, %**
$k_{e0,hemin}$, h^{-1}	0.00764	15.6	0.00807	16.5	0.00761	14.8
Slope, mL/μg	0.035	18.0	0.0328	20.9	0.034	19.9
$I_{max,givo}$	0.966	22.6	0.931	26.5	0.956	12.3
$IC_{50,givo}$ (AHP), ng/g	0.587[a]	2.19	0.444[a]	2.36	0.476[a]	2.43
$IC_{50,givo}$ (CHE), ng/g	0.177[a]	2.72	0.093[a]	3.28	0.100[a]	3.40
$k_{in,ALA}$ (AHP), mmol/mol Cr·h	11.82	3.02	9.68	7.32	11.47	3.14
$k_{in,ALA}$ (CHE), mmol/mol Cr·h	5.003	9.13	4.437	13.00	5.312	8.41
$k_{out,ALA}$, h^{-1}	0.84 fixed[b]		0.84 fixed[b]		0.84 fixed[b]	
Change in log($I_{max,givo}$) per						
log(Baseline ALA)			0.0324	23.8	0.0368	20.5
log(age)			−0.0412	41.5		
Fraction change in $I_{max,givo}$ for						
Mild hepatic impairment			1.01	145.0		
Mild renal impairment			1.02	72.2		
Moderate renal impairment			1.03	55.4		
Change in log($k_{in,ALA}$) per log(age)			0.0402	140.4		
Fraction change in $k_{in,ALA}$ for						
Mild hepatic impairment			1.5	36.4	1.41[c]	45.8[c]
Mild renal impairment			1.25	81.6		
Moderate renal impairment			1.24	81.7		
Residual error	52.9%	3.44	52.3%	3.57	52.4%	3.60
OFV	25.153		−49.686		−28.827	

Abbreviations: AHP, acute hepatic porphyria; CHE, chronic high excreters; CI, confidence interval; CL, clearance; $IC_{50,givo}$, givosiran concentration in RISC required to reach 50% of maximum inhibition; IIV, interindividual variability; $I_{max,givo}$, maximum inhibitory effect of givosiran on $k_{in,ALA}$; k_a, uptake rate constant to liver; $k_{e0,hemin}$, effect compartment equilibrium rate constant of hemin; $k_{in,ALA}$, zero-order synthesis rate constant for ALA; $k_{out,ALA}$, first-order degradation rate constant for ALA; OFV, objective function value; Q, intercompartmental clearance; RSE, relative standard error; V_1, volume of distribution of central liver compartment; V_2, volume of distribution of peripheral liver compartment.

[a]Values were converted from μg/g to ng/g.

[b]The $k_{out,ALA}$ value for ALA was fixed to 0.84 h^{-1} from literature.[23]

[c]Patients with moderate or severe hepatic impairment were included in the mild hepatic impairment category.

constant between the plasma and effect compartments was estimated to be 0.00761 h^{-1} (half-life, 91 h). The IIVs for slope, $IC_{50,givo}$, and $k_{in,ALA}$ were 59.0%, 144%, and 68.7%, respectively, and residual variability was 52.4%.

Participants with CHE were more sensitive to the ALA-lowering effects of givosiran and its metabolite than patients with AHP (Figure 2). Covariate evaluation demonstrated a higher $IC_{50,givo}$ in patients with AHP (0.476 ng/g) than in participants with CHE (0.100 ng/g). Baseline ALA was a significant covariate on $I_{max,givo}$, with patients who had lower baseline values having slightly lower I_{max} (Figure 2). However, differences in ALA lowering at various baseline ALA levels were minimal, with the model predicting steady-state reductions in urinary ALA of 88.3%, 92.3%, and 94.5% from baseline in patients with baseline ALA levels four times the upper limit of normal (ULN; the protocol-defined entry criterion for urinary ALA [1.47 mmol/mol Cr]), 10 × ULN, and 25 × ULN, respectively. Baseline hepatic impairment status was a significant covariate on $k_{in,ALA}$ (Figure 2); patients with mild hepatic impairment had 40% higher baseline ALA levels relative to patients with normal hepatic function.

Model evaluation

The final population PK/PD model provided reasonable fits for observed ALA data. Population and individual predictions were consistent with the observed data, with acceptable central tendencies (Figure S2). Visual predictive checks of the final model explained the observed data well, with the 95% prediction intervals of median, 10th, and 90th percentiles encompassing observed median, 10th, and 90th percentiles in the double-blind period of ALN-AS1-003. The model adequately captured variability in the data and the time course of PD response following givosiran administration. Further, the observed ALA and model-predicted ALA response at 3 and 6 months from ALN-AS1-003 are in close agreement, thus supporting the adequacy of the model in describing the PD effects of givosiran (Figure 3).

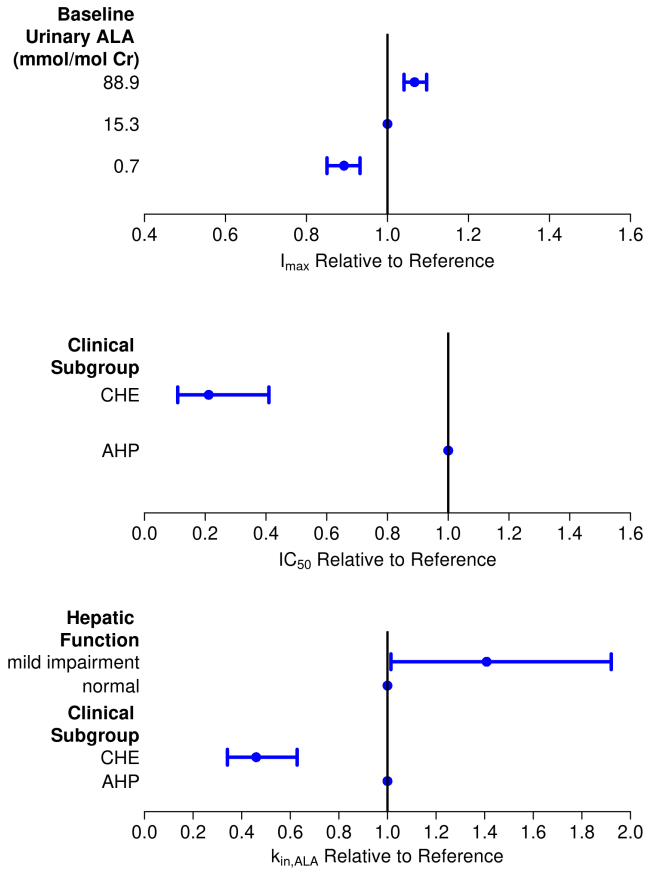

FIGURE 2 Covariate effects on I_{max}, IC_{50}, and $k_{in,ALA}$ in the final population pharmacokinetic/pharmacodynamic model. Data are medians and 95% confidence intervals (CIs). ALA, aminolevulinic acid; AHP, acute hepatic porphyria; CHE, chronic high excreter; Cr, creatinine; IC_{50}, concentration required to produce half-maximal effect of givosiran; I_{max}, maximum inhibitory effect of givosiran on $k_{in,ALA}$; $k_{in,ALA}$, zero-order synthesis rate of ALA.

Model application

Simulations following monthly dosing demonstrated dose-dependent lowering of urinary ALA in patients with AHP (data not shown). At steady-state, givosiran 2.5 mg/kg q.m. is predicted to reduce median urinary ALA level from 15.3 to 1.37 mmol/mol Cr, reflecting a 90.5% reduction from baseline. This translates to 52.3% of patients with steady-state urinary ALA levels less than 1 × ULN (1.47 mmol/mol Cr) and 87.9% of patients with steady-state ALA levels less than 3 × ULN. Doubling the givosiran dose from 2.5 to 5 mg/kg q.m. is predicted to result in minimal incremental ALA lowering, with median urinary ALA level estimated to decrease from 1.37 to 1.08 mmol/mol Cr, a 92.5% reduction from baseline. Decreasing the givosiran dose to 1.25 mg/kg q.m. resulted in estimated median urinary ALA of 1.87 mmol/mol Cr, an 86.9% reduction from baseline.

Urinary ALA reduction with q3m dosing is predicted to be less than with q.m. dosing (Figure 4). Furthermore, substantial fluctuations in urinary ALA level are observed during q3m dosing, with levels trending back toward baseline at the end of the dosing interval. Givosiran 5 mg/kg q3m is predicted to reduce urinary ALA to below the ULN for only 32.0% of patients compared with 52.3% for givosiran 2.5 mg/kg q.m., demonstrating that a higher quarterly dose is not as effective as a monthly dosing regimen for maintaining maximum suppression of ALA over the dosing interval.

Simulations revealed that ALA levels and percent reduction in ALA at steady-state were similar across the range of body weights observed in clinical studies. PD response in patients weighing 40 kg and 130 kg (the lowest and highest body weights across studies) were comparable with that of patients weighing 66.2 kg (median body weight). Median urinary ALA level and reduction from baseline in ALA with givosiran 2.5 mg/kg q.m. were predicted to be similar for adult and adolescent patients with AHP (Figure 5).

DISCUSSION

Using pooled data from phase I–III clinical trials of givosiran, a population PK/PD model was developed to describe the relationship between givosiran exposure and PD response, as measured by change in urinary ALA level. The semimechanistic model correlates predicted active siRNA concentrations in liver and RISC with urinary ALA levels and adequately describes the time course of urinary ALA reduction after givosiran administration, the magnitude of PD effect and IIV across a wide range of givosiran doses (0.035–5 mg/kg) and dosing regimens, and the influence of differences in clinical subgroup.

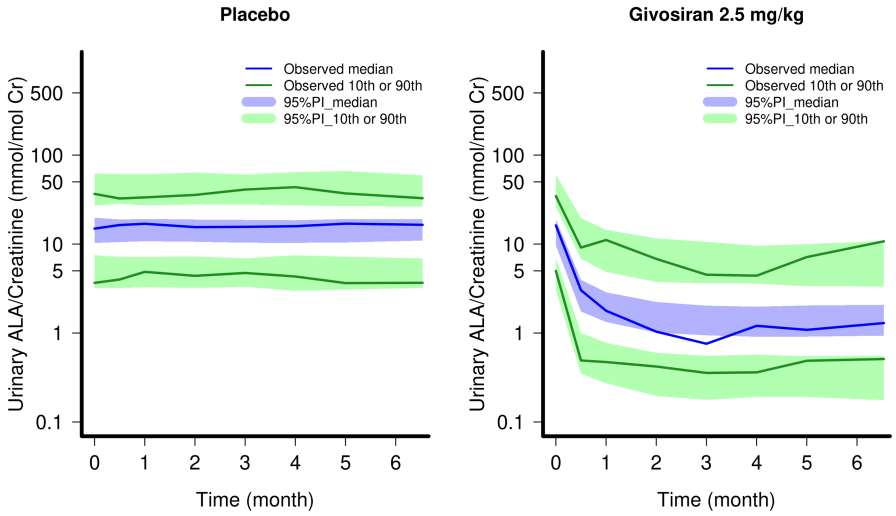

FIGURE 3 Visual predictive checks for observed and model-predicted urinary ALA concentration in patients with acute hepatic porphyria. Data from patients who received placebo or givosiran 2.5 mg/kg once monthly during the double-blind treatment period of study ALN-AS1-003. ALA, aminolevulinic acid; Cr, creatinine; PI, prediction interval.

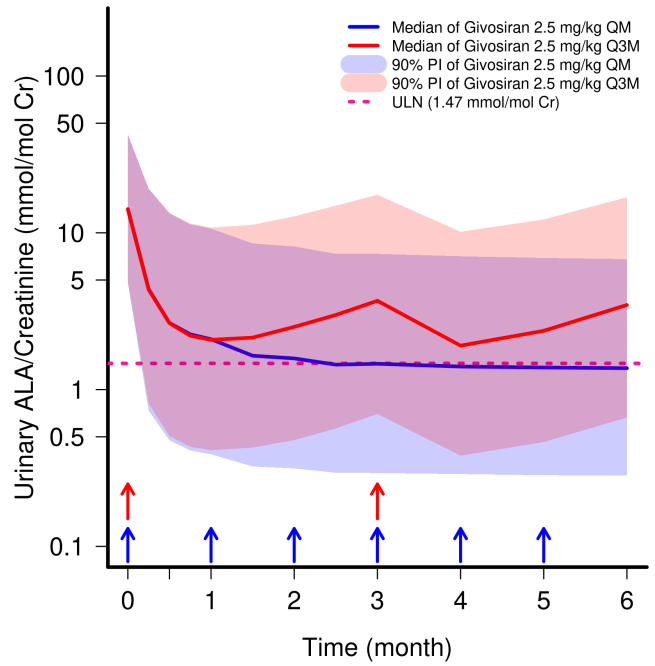

FIGURE 4 Model-predicted urinary ALA concentration versus time profiles after givosiran 2.5 mg/kg administered once monthly (QM) or once quarterly (Q3M) to patients with acute hepatic porphyria. Blue arrows represent dose timing for QM givosiran; red arrows represent dose timing for Q3M givosiran. ALA, aminolevulinic acid; Cr, creatinine; PI, prediction interval; ULN, upper limit of normal.

A key finding of this analysis was validation of the approved givosiran dosing regimen for the treatment of AHP (2.5 mg/kg q.m.)[27,28] as optimal for achieving and maintaining suppression of ALA at close-to-normal levels. Simulations derived from the model demonstrated that givosiran 2.5 mg/kg q.m. provides meaningful lowering of urinary ALA below ULN for 52.3% of patients. Predicted ALA reduction with givosiran 2.5 mg/kg q.m. at steady-state was 90.5%. Increasing the dose to 5 mg/kg yields comparable ALA lowering to that of the 2.5-mg/kg dose, indicating that the 2.5-mg/kg dose is in the plateau portion of the dose–response curve. Doses lower than 2.5 mg/kg are predicted to result in reduced effect, with a lower proportion of patients attaining normalization of ALA. Similarly, extending the interval between doses from q.m. to q3m, even at higher doses, is predicted to reduce achievement of urinary ALA normalization and increase peak-trough fluctuation.

The model also revealed that the givosiran exposure-response relationship was not significantly influenced by body weight, age, sex, ethnicity (East Asian vs. non-East Asian), or renal function. Simulations from the model indicated that body weight-based dosing (2.5 mg/kg) yields similar ALA-lowering effects across a large spectrum of body weights (40–130 kg). Covariates that were statistically significant and retained in the final model included baseline ALA, clinical subgroup (CHE vs. AHP), and mild hepatic impairment. The association between lower baseline ALA and lower $I_{max,givo}$ is likely due to a floor effect, whereas the lower $IC_{50,givo}$ for participants with CHE versus patients with AHP is potentially related to greater ALAS1 induction in the latter group.[5] Higher levels of baseline ALA in patients with AHP compared with participants with CHE indicates an association between baseline ALA levels and disease severity. Greater ALAS1 induction in AHP required q.m. injections of givosiran to attain sustained reductions in ALA levels to near normal range, whereas less frequent injections were required in CHE to achieve the same. Mild hepatic impairment was associated with higher (40%) baseline ALA levels; however, there were no differences in givosiran-mediated ALA lowering in patients with mild hepatic impairment compared with those who have normal hepatic function.

Additionally, on-treatment median urinary ALA levels and percent reduction from baseline were predicted to be comparable between adult (≥18 years) and adolescent (≥12 to <18 years) patients with AHP who receive givosiran

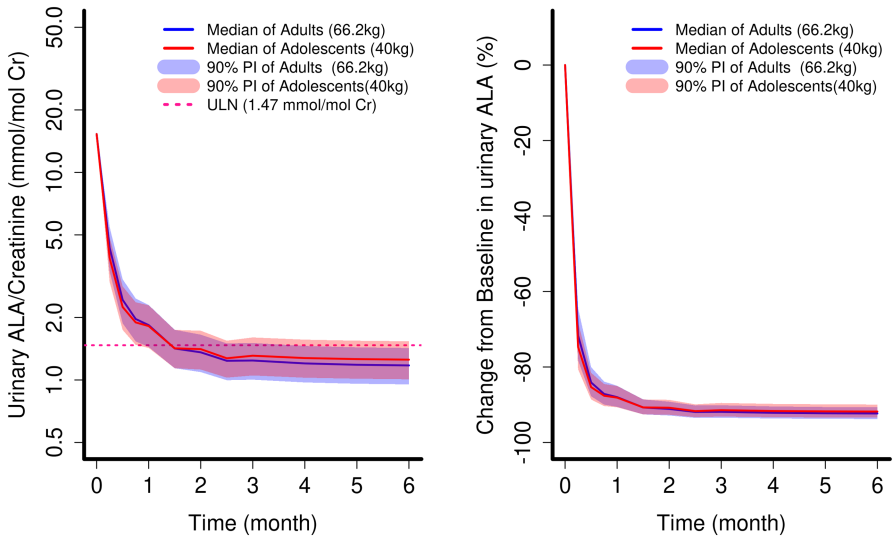

FIGURE 5 Model-predicted urinary ALA concentration versus time profiles after administration of givosiran 2.5 mg/kg once monthly to adolescent (age ≥12 to <18 years) and adult (age ≥18 years) patients with acute hepatic porphyria. Data are model-predicted absolute concentrations (left panel) and percent change from baseline (right panel) over time. ALA, aminolevulinic acid; PI, prediction interval; ULN, upper limit of normal.

2.5 mg/kg q.m. Overall, no dose adjustment from the approved dose would be required for any of the evaluated subgroups.

The PK/PD model described herein is unique in several respects. First, plasma PKs do not constitute "exposure" with regard to developing the exposure-response relationship in this model because givosiran is rapidly removed from systemic circulation, with plasma concentrations falling below the LLOQ within 24 h postdose.[15] The residence time of active siRNA in the liver, however, is substantially longer, as confirmed in nonclinical studies.[13,22] Second, it is the siRNA concentration loaded onto RISC in the hepatocytes that mediates specific cleavage of target mRNA, leading to reduction in *ALAS1* mRNA and, consequently, ALAS1 protein. Therefore, the PK of givosiran in the model was described by modeling the uptake of givosiran in the liver and subsequent loading of siRNA onto the RISC complex. Data from nonclinical studies across GalNAc-conjugated siRNAs, including givosiran, show that RISC-loaded siRNA levels represent effective concentrations driving the PD response in the liver.[22] Nonclinical data have also shown that givosiran liver PKs and RISC loading are linear within a 0.3–10 mg/kg dose range[22]; therefore, linear kinetics was used to describe liver and RISC PKs and PDs and for allometrically scaling to humans. In nonclinical PK/PD studies, the time course of *ALAS1* mRNA silencing correlated with the kinetics of RISC-loaded siRNA levels, with a similar peak time and slow return to baseline.[29] This is supported by the time course of *ALAS1* reduction in humans, where peak reduction is achieved ~4 weeks after dosing,[5] whereas liver givosiran concentrations are expected to peak within a day after s.c. dosing based on the rapid disappearance of drug from plasma. Thus, RISC-loaded siRNA levels peak slowly over weeks after dosing and represents the relevant exposure driving the time course of PD response in the liver. As it is not feasible to measure RISC-loaded active siRNA concentrations in humans, these concentrations were predicted from the nonclinical PK/PD model. Last, due to the effects of hemin on urinary ALA, the PK/PD model needed to account for the effect of hemin administration.

In summary, the PK/PD model adequately characterized the exposure-response relationship for givosiran. None of the covariates tested had a clinically relevant effect on PD response that would necessitate dose adjustment. For patients with AHP, including adults, adolescents, and patients with mild or moderate renal impairment or mild hepatic impairment, the 2.5-mg/kg q.m. dosing regimen of givosiran is expected to produce clinically meaningful ALA lowering, thus reducing the risk for AHP attacks.

AUTHOR CONTRIBUTIONS

J.L., M.M., and G.J.R. wrote the manuscript. G.J.R. designed the research. J.L. performed the research. J.L., M.M., and G.J.R. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

J.L., M.M., and G.J.R. are Alnylam Pharmaceutical employees and hold Alnylam stocks or options.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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